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Chemical components and health-promoting properties of wheat fermented with *Schizophyllum commune* mushroom

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Abstract
Schizophyllum commune, an underutilized mushroom known for its diverse health-promoting components, was utilized in solid-state fermentation (SSF) of wheat grains to produce myceliated products. Such products have gained remarkable interest as potential functional foods. The present study aimed to develop an *S. commune*-fermented wheat product and investigate the effects of SSF on its chemical profile and health-promoting properties. Wheat grains were inoculated with *S. commune* mycelium and incubated for 30 days. Nutritional composition, functional properties, and chemical profiles were determined using established methodologies. The results revealed that fermentation with *S. commune* significantly altered the protein, carbohydrate, vitamin and mineral contents of wheat. Notably, the total phenolic and flavonoid contents of the fermented wheat improved by up to 3.4-fold and 1.8-fold, respectively. Antioxidative potential was evaluated using DPPH radical scavenging, ferric-reducing, and β-carotene bleaching inhibition assays. The water extracts from the fermented wheat exhibited superior activity compared to the other solvent extracts. Furthermore, the anti-inflammatory properties of the fermented wheat were significantly enhanced relative to its unfermented counterpart, as evidenced by its capacity to scavenge nitric oxide and inhibit protein denaturation. Untargeted LC-QTOF MS analysis of the cold-water extract, which exhibited the highest bioactivity led to the putative identification of 70 metabolites, including phenolic acids, flavonoids, and organic acids. The presence of compounds such as gallic acid, astragalin, and glucuronic acid suggests a synergistic contribution to the observed health-promoting effects. These findings indicate that *S. commune*-fermented wheat serves as a promising functional food ingredient, offering a sustainable candidate for the development of products aimed at promoting consumer health and wellness.

Keywords Myceliated grain, Solid-state fermentation, Nutrition, Antioxidant, Anti-inflammatory, Bioactive compounds

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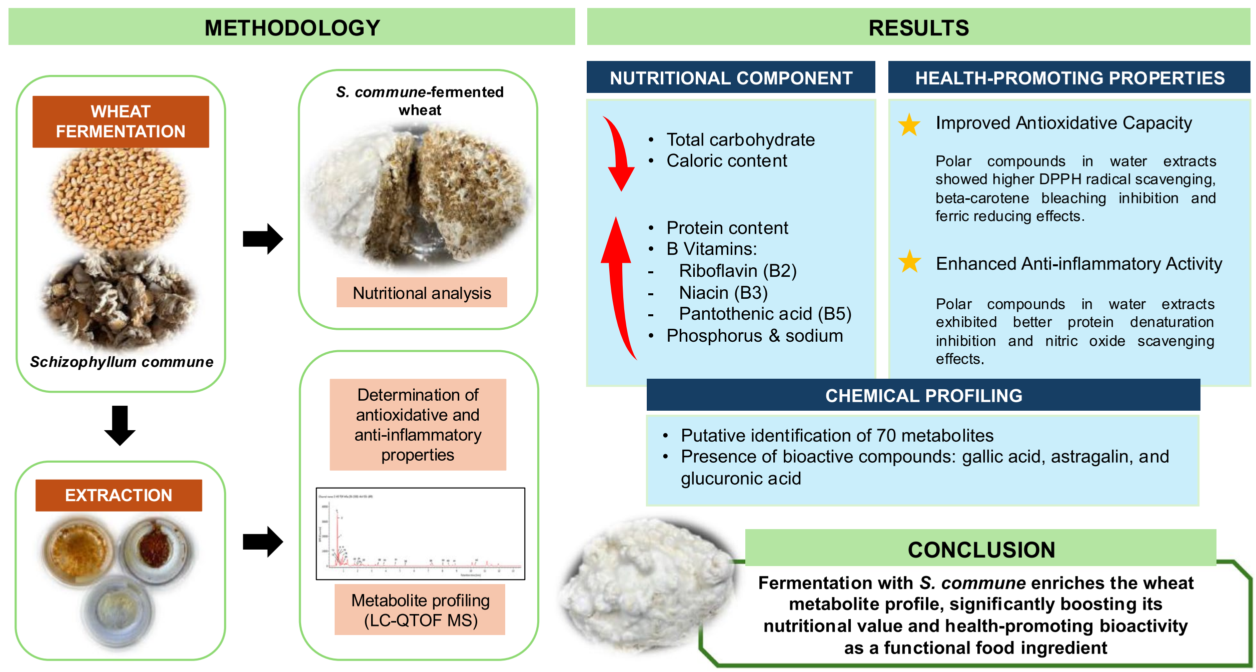
Graphical Abstract



Introduction

Functional foods, nutraceuticals, and dietary supplements are highly sought after by health-conscious consumers to promote wellness and mitigate the risk of non-communicable diseases (NCDs). Considering the immediate need to address food security and improve the global nutritional sustainability, mushrooms have gained significant scientific focus as functional foods and nutraceutical sources. Consequently, their intake in diverse forms and formulations is being actively explored. Mushrooms have transitioned from niche ingredients to significant global food players, driven by food technologies such as fermentation and extrusion, which improve the texture and flavour of mushroom-based products (Cantato & Conte-Junior, 2025; Di Cianni et al., 2023). Mushroom mycelium, an environmentally sustainable material, is nutritious and contains consortia of bioactive compounds comparable to those of mushroom fruit bodies (Holt et al., 2024; Sułkowska-Ziaja et al., 2025). With the current rapid progress in mycelium-based food products, owing to their unique flavour, nutritional and health-promoting values, ecological credentials, and sustainability, mushroom mycelia are currently hailed as future foods (Holt et al., 2024). Mycelium produced from solid-state fermentation (SSF) has the potential to be incorporated into food products, improving their functionalities and nutritional attributes and ultimately refining their sensorial properties (Antunes et al., 2020). In the SSF method, mycelia are typically grown on grain substrates, and these fermented substrates, also known as myceliated grains, can be developed directly into food products due to the enhanced nutritional content and biological functionalities resulting from fermentation process (Benson et al., 2019). As potential functional foods and novel probiotic delivery vehicles, fermented cereal grain products are well suited for individuals with lactose intolerance or those adhering to a low-fat or vegan diets (Tsafrakidou et al., 2020).

One mushroom species with significant potential for exploitation is *Schizophyllum commune*, a basidiomycete distributed worldwide. Owing to its edibility and medicinal properties, *S. commune* is currently regarded as one of the most important mushrooms, and its popularity is increasing. Although *S. commune* is easily cultivated using low-cost agricultural byproducts (Debnath et al., 2020), it is relatively underutilized compared to other popular mushrooms, such as *Pleurotus* spp., and research regarding its health-promoting components remains limited (Mišković et al., 2023). While numerous myceliated grain powders are marketed as health supplements, no research to date has specifically investigated the use of *S. commune* mycelia and their fermented substrate as a source for nutraceuticals and functional ingredients. Wheat (*Triticum aestivum* L.) is one of the most widely consumed grains and is also used for the established production of mushroom spawn (Silva et al., 2024). Several studies have reported the enhancement of the nutritional content, sensory attributes, and functional features of wheat through fermentation (Zhang et al., 2022); however, comprehensive studies on the chemical profile and health-promoting properties of mushroom-fermented wheat remain limited.

As the relationship between NCDs and oxidative stress is increasingly well acknowledged, antioxidants and anti-inflammatory therapies are being intensively investigated as defenses against various pathologies (Arulselvan et al., 2016; Grosso, 2018). The modern food and nutraceutical industries are exploring sustainable resources that can accommodate the necessities of the human diet in terms of nutritional quality while also strengthening the overall health of consumers (Vignesh et al., 2024). The present study aimed to produce *S. commune*-fermented wheat products and evaluate their health-promoting properties and chemical constituents. To promote the sustainable use of natural resources, these fermented products were subjected to multi-solvent extraction to evaluate their antioxidative and anti-inflammatory potential. To the best of our knowledge, this study is the first to report the chemical components and anti-inflammatory activities of *S. commune*-fermented wheat, thereby adding a novel dimension to the documented antioxidant properties of this mushroom species.

Materials and methods

Chemicals

Potato dextrose agar and potato dextrose broth were obtained from Oxoid. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), sodium nitroprusside ($Na_2[Fe(CN)5NO]$), sodium chloride (NaCl), aluminum chloride ($AlCl_3$), ascorbic acid, Trolox, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), iron chloride hexahydrate ($FeCl_3$), sodium acetate trihydrate, gallic acid, bovine serum albumin (BSA) and diclofenac sodium were obtained from Sigma Aldrich (USA). Folin-Ciocalteu reagent was purchased from Merck (Germany), while Tween 20 was purchased from Amresco (OH, USA). All reagents and chemicals used were of analytical grade. Hexane, chloroform, ethyl acetate, absolute ethanol, methanol, 85% phosphoric acid and sulfuric acid (H_2SO_4) were of analytical grade and were obtained from J.T. Baker (USA) and Fisher Scientific (UK).

Mycelial liquid culture preparation

Schizophyllum commune mother culture (MARDI SC1) was obtained from the Malaysian Agricultural Research and Development Institute (MARDI) collection. The *S. commune* mycelium was maintained in slant tubes at 4 °C and cultured in Petri dishes at 25 °C on potato dextrose agar until it was used to produce the mycelial liquid culture. Potato dextrose broth (20 g/L) was used as the basal medium for the growth of *S. commune* mycelia. One hundred fifty milliliters of the basal medium was poured into conical flasks and autoclaved for 21 min at 121 °C (Hirayama, Japan). A 1 cm diameter mycelial culture from the *S. commune* dish was used to inoculate the sterilized medium. The medium was shaken at 150 rpm and incubated at 28 ± 1 °C for 7 days.

Solid-state fermentation (SSF)

The wheat (*T. aestivum* L.) used in this study was food grade and was supplied by FFM Berhad, Selangor, Malaysia. Upon arrival at the lab, the wheat was contained in a food-grade polypropylene plastic bag. The fermentation of wheat was carried out according to the method outlined by Krishnen et al. (2021). The wheat grains were washed, soaked in hot boiling water for 1 h, and left to cool before being packed (200 g each) into a polypropylene plastic bag (30 cm × 10 cm × 10 cm). The moisture content of all the samples was adjusted to 50 to 55% using a moisture analyzer and autoclaved for 21 min at 121 °C. Prior to inoculation, the mycelial liquid culture was homogenized with a sterile homogenizer for 30 s to ensure a uniform and consistent distribution of mycelial fragments. The sterilized wheat grain was then inoculated with 5 mL of the homogenized liquid culture with a sterile pipettor under aseptic conditions and incubated for 30 days at 28 ± 2 °C in the dark. The entire contents of the bags were lyophilized. All the dried unfermented and fermented samples were milled and sieved through a 0.3 mm mesh.

Analysis of nutritional components

Proximate analysis

Protein, total fat, ash, and dietary fibre contents were determined according to analytical methods based on AOAC 2005. Calories and total carbohydrates were calculated on the basis of the method of analysis for nutrition labelling, AOAC 1993. Total sugars were determined via high-performance liquid chromatography coupled with a refractive index detector (HPLC-RID). All determinations were performed by an ISO/IEC 17025 accredited laboratory (SGS Malaysia Sdn. Bhd.).

Vitamin and mineral contents

The vitamin content was determined using in-house methods involving high-performance liquid chromatography coupled with a fluorescence detector (HPLC-FLD). The determination of minerals was performed according to in-house methods based on AOAC 986.15, 975.03 & 922.02, APHA3120B and APHA 3125B (ICP–OES). All analyses were performed by an ISO/IEC 17025 accredited laboratory (SGS Malaysia Sdn. Bhd.).

Beta-glucan content

Determination of the β-glucan content was performed using a β-glucan assay kit (Megazyme, Ireland). The assay was performed in accordance with the manufacturer's protocols.

Preparation of crude extracts

For hot water extraction, fermented and unfermented wheat powder was extracted with hot distilled water (1:10 w/v) at 80 °C for 60 min in a shaking water bath (100 rpm). For cold water extraction, the samples were extracted with cold distilled water (1:10 w/v) at 4 °C for 60 min in a refrigerated shaking incubator (100 rpm). A 60-min extraction time was standardized for both cold and hot water to compare the effects of temperature on bioactive compound extractability by water. For organic solvent extraction, the samples were extracted with ethanol, ethyl acetate or hexane (1:10 w/v) at 25 °C for 24 h in a refrigerated shaking incubator (150 rpm). Each extracted slurry was centrifuged (1000 rpm, 10 min) and filtered (Whatman No. 1) to collect the supernatant. The insoluble residue was treated again twice as described above. The pooled supernatants of the water extracts were lyophilized, while the pooled supernatants of the organic solvent extracts were concentrated by using a rotary evaporator (Buchi, Switzerland). The yield percentage was calculated as follows:

$$\text{Yield percentage}(\%) = (\text{weight of crude extract}/\text{weight of samples}) \times 100\%$$

For the biological assays, 10 mg/mL extract solution was prepared by diluting the dried extract in water (for the crude water extracts) and ethanol (for the organic solvent extracts).

Determination of polyphenolic components

Total phenolic content

The total phenolic content was measured according to the methods of Abd Razak et al. (2020). Each sample (1 mL) was added to 5 mL of Folin-Ciocalteu reagent (Merck, USA). The mixture was subsequently combined with 4 mL of sodium carbonate (7.5%) and allowed to react in the dark for 120 min. A UV–Vis spectrophotometer was used to measure the absorbance at 765 nm. The calibration curve was plotted using gallic acid as a reference standard, and the result was expressed as µg gallic acid equivalent (GAE)/mg extract.

Total flavonoid content

The total flavonoid content was measured with reference to the method of Abd Razak et al. (2019). Briefly, 1 mL of extract was combined with distilled water (4 mL) and 5% $NaNO_2$ solution (0.3 mL). After 5 min of incubation, 10% $AlCl_3$ (0.3 mL) was added, and the mixture was left to stand for 6 min. Subsequently, 1 M NaOH solution (2 mL) was added, and the final volume of the mixture was adjusted to 10 mL with double-distilled water. The mixture was allowed to react for 15 min, after which the absorbance (430 nm) was measured. The reference standard used was quercetin, and the result was expressed as µg quercetin equivalent (QE)/mg extract.

Antioxidative property assay

DPPH (diphenyl-1-picryl-hydrazyl) radical scavenging activity

The assay was carried out according to a method described by Bobo-García et al. (2015) with slight modifications. The samples (20 µL) in a 96-well plate were mixed with 180 µL of freshly prepared DPPH solution (150 µmol/L in 80:20 v/v methanol:water) and shaken for 60 s. Following incubation in the dark for 40 min at room temperature, the absorbance was measured at 515 nm with a microplate reader. Ascorbic acid and Trolox (0.1 mg/mL) were used as positive controls in this assay. The following equation was used to determine the percentage of scavenged DPPH:

$$\text{DPPH radical scavenging activity}(\%) = [1-(A_{sample}-A_{blank})/(A_{control}-A_{blank})]\times 100$$

where A_{sample} is the absorbance of a sample with the DPPH solution, A_{blank} is the absorbance of water or solvent with methanol–water mixture, and $A_{control}$ is the absorbance of water or solvent with the DPPH mixture.

Ferric-reducing antioxidant property (FRAP)

A previously described procedure (Abd Razak et al., 2020) was followed. In this study, the FRAP reagent was prepared by combining 300 mM acetate buffer, 10 mM TPTZ and 20 mM $FeCl_3$, where 10 parts buffer were mixed with one part TPTZ and one part $FeCl_3$. An aliquot (150 µL) of the sample was reacted with 2850 µL of fresh FRAP reagent for 30 min in the dark. The absorbance of the mixtures at 593 nm was recorded using a UV–Vis spectrophotometer. The standard curve was constructed using 10—1000 µM $FeSO_4$ solution, and the result was expressed as µM ferrous equivalent (FE)/mg extract.

Beta-carotene bleaching inhibition activity

This assay was conducted according to the methods of Kassim et al. (2013). In summary, a total of 2100 µL of β-carotene solution (1 mg/mL in chloroform) was mixed with 50 µL of linoleic acid and 420 µL of Tween 20 in a flask. The chloroform was removed via a rotary evaporator. Afterwards, distilled water (100 mL) was introduced with vigorous agitation to create an emulsion. The emulsion (200 µL) was added to each of the 96-well microplates containing the test samples (50 µL). Ascorbic acid and Trolox (1 mg/mL) were used as positive controls. A blank was prepared using a reaction mixture without β-carotene and test samples, whereas a control was prepared using an emulsion without test samples. The samples' absorbance (at 470 nm) was read promptly at the beginning and after a 2 h incubation period at 50 °C in the dark. The antioxidant activity (AA) was quantified by assessing its ability to prevent β-carotene bleaching effectively, as determined by the following formula:

$$\text{Antioxidant activity}(\%) = [1-(\text{Absorbance}_{t=0}-\text{Absorbance}_{t=2})/(\text{Absorbance}_{c=0}-\text{Absorbance}_{c=2})]\times 100$$

where Absorbance $_{t=0}$ and Absorbance $_{t=2}$ represent the absorbances of the test samples measured at 0 and 2 h, respectively. The absorbance $_{c=0}$ and the absorbance $_{c=2}$ represent the absorbance of the control measured at 0 and 2 h, respectively.

Anti-inflammatory property assay

Nitric oxide scavenging activity

The assay was performed according to the methods of Abd Ghafar et al. (2018), with some modifications. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid. A volume of 50 µL of sodium nitroprusside (10 mM) was mixed with 100 µL of *S. commune*-fermented wheat extracts in a 96-well plate and incubated under light for 3 h. The reacted mixtures were transferred into a new 96-well plate and mixed with an equal volume of Griess reagent. The absorbance was measured at 546 nm, and gallic acid (1 mg/mL) was used as a positive control. The nitrite radical scavenging activity of the samples was calculated according to the following formula:

$$\text{Nitric oxide scavenging activity}(\%) = [(A_{control} - A_{sample})/(A_{control})] \times 100$$

where A $_{sample}$ is the absorbance of a sample with the assay reagent, and A $_{control}$ is the absorbance of water or solvent with the assay reagent.

Inhibition of protein denaturation activity

A bovine serum albumin (BSA) denaturation assay was carried out according to the methods of Nirmal and Panichayupakaranant (2015), with modifications. A stock solution of 1% (w/v) BSA was prepared in phosphate-buffered saline (pH 6.4). The reaction mixture consisted of 2850 µL of BSA solution and 150 µL of test samples prepared in test tubes. The tubes were incubated at 37 °C in a water bath for 20 min, followed by incubation at 72 °C for 20 min. After cooling, the absorbance of the mixtures was determined with a spectrophotometer at 660 nm. Diclofenac sodium (1 mg/mL) was used as a standard non-steroidal anti-inflammatory drug (NSAID) reference, and water and solvent were used as controls. The inhibition of protein denaturation was calculated via the following formula:

$$\text{Inhibition of protein denaturation}(\%) = [1 - (A_{sample}/A_{control})] \times 100$$

where A $_{sample}$ is the absorbance of a sample with the BSA solution and A $_{control}$ is the absorbance of water or solvent with the BSA solution.

LC-QTOF MS analysis

Ultrahigh-performance liquid chromatography (UHPLC) was conducted via an ACQUITY UPLC I-Class system from Waters, which comprises a binary pump, a vacuum degasser, an autosampler, and a column oven. The compounds were chromatographically separated via an ACQUITY

Table 1 Proximate composition of unfermented and *S. commune*-fermented wheat on a dry weight basis

Parameter	Unfermented wheat	*S. commune*-fermented wheat
Moisture (%)	0.32 ± 0.03[b]	2.05 ± 0.03[a]
Carbohydrate (g/100 g)	80.10 ± 0.85[a]	70.40 ± 0.85[b]
Fat (g/100 g)	2.90 ± 0.85[a]	2.95 ± 0.35[a]
Protein (g/100 g)	10.00 ± 0.14[b]	12.65 ± 0.07[a]
Ash (g/100 g)	1.40 ± 0.28[a]	1.55 ± 0.07[a]
Dietary fibre (g/100 g)	11.85 ± 1.20[a]	14.05 ± 0.35[a]
Total sugars (g/100 g)	0.55 ± 0.07[a]	1.48 ± 1.21[a]
Energy (kcal/100 g)	386.50 ± 3.54[a]	358.50 ± 0.71[b]

The values are the means of duplicate determinations ($n = 2$) ± standard deviations

Different letters in the same row indicate statistically significant differences ($p < 0.05$)

UPLC HSS T3 column (100 mm × 2.1 mm × 1.8 µm) from Waters operated at 40 °C. A linear binary gradient of water (0.1% formic acid) served as mobile phase A, while acetonitrile constituted mobile phase B. The content of the mobile phase was modified throughout the run as follows: at 0 min, 1% B; at 0.5 min, 1% B; at 16.00 min, 35% B; at 18.00 min, 100% B; and at 20.00 min, 1% B. The flow rate was 0.6 mL/min, and the injection volume was 1 µL.

The UHPLC system was integrated with a Vion IMS quadrupole time–of–flight (QTOF) hybrid mass spectrometer from Waters, featuring a Lock Spray ion source. The ion source functioned in negative electrospray ionization (ESI) mode under the following precise conditions: capillary voltage, 1.50 kV; reference capillary voltage, 3.00 kV; source temperature, 120 °C; desolvation gas temperature, 550 °C; desolvation gas flow, 800 L/h; and cone gas flow, 50 L/h. Nitrogen (> 99.5%) was utilized as the desolvation and cone gas. Data were collected in high-definition MSE (HDMSE) mode within the m/z range of 50–1500 at a rate of 0.1 s each scan. Consequently, two distinct scans with varying collision energies (CEs) were alternately obtained during the experiment: a low-energy (LE) scan at a constant CE of 4 eV and a high-energy (HE) scan in which the CE was incrementally adjusted from 10 to 40 eV. Argon (99.999%) served as the collision-induced dissociation (CID) gas. The data were analysed via Waters UNIFI Qualitative and Quantitative software with a Waters natural products library.

Statistical analysis

All the results (except for those for nutritional components) were the average of three independent assays performed in triplicate and are presented as the means ± standard deviations (SDs). The data were statistically analysed by one-way analysis of variance (ANOVA) and Tukey's post hoc test via Minitab software, version 14. A value of $p < 0.05$ was considered statistically significant.

Results and discussion

Nutritional components of unfermented and *S. commune*-fermented wheat

As a traditional industrial procedure, the beneficial effects of SSF on the nutritional composition of raw materials are mostly dependent on the ability of the microorganisms involved in this bioprocess to produce the necessary nutrients for their growth from the substrate in which they reproduce. For example, during fermentation of raw materials, fungi release several types of enzymes, such as amylases, xylanases, and cellulases, that degrade plant cell walls and subsequently improve the digestibility of fermented products (Sadh et al., 2018). The nutritional absorption process of mushroom mycelia is influenced by various factors, such as species, substrates, and enzymes, which contribute to the variations in nutrient utilization observed among the different mushrooms (Espinosa-Paez et al., 2017).

Fermentation with *S. commune* led to distinct shifts in the proximate composition of wheat (Table 1). Specifically, the carbohydrate content decreased significantly, while the crude protein content increased following fermentation. Similar trends were observed in SSF studies involving *Sanghuangporus sanghuang* (Song et al., 2021), using rice and barley as substrates. In that study, the carbohydrate content of rice decreased from 89% to 84.77%, with a corresponding increase in crude protein from 9.26 g/100 g to 12.41 g/100 g. For barley, carbohydrate decreased from 85.27% to 75.47%, and the protein content increased substantially from 10.41 g/100 g to 18.83 g/100 g. Additionally, the crude protein content of soybean meal increased from 48.39% to 52.98% after fermentation with *P. ostreatus*, to 51.95% with *Hericium erinaceus*, and to 51.18% with *Flammulina velutipes* (Wang et al., 2023). These quantitative and qualitative shifts in protein content are largely attributable to the loss of dry matter content, primarily carbohydrates, during fermentation. Carbohydrates are used as a fuel source by microorganisms, and carbon dioxide is produced as a byproduct. As a result, the nitrogen in fermented substrates is concentrated, which increases the proportion of protein (Cui et al., 2012). Furthermore, the observed decrease in carbohydrate content and increase in protein content may be attributed to the sophisticated enzyme system of *S. commune*. This system involves the active production and use of carbohydrate-active enzymes (CAZymes), such as β-glucosidase, during *S. commune*'s

Table 2 Vitamin and mineral composition and beta-glucan content of unfermented and *S. commune*-fermented wheat on a dry weight basis

Parameter (mg/100 g)	Unfermented wheat	*S. commune*-fermented wheat
Vitamins (mg/100 g)		
Thiamine (B1)	0.10 ± 0.05[a]	ND
Riboflavin (B2)	ND	0.09 ± 0.00[a]
Niacin (B3)	0.76 ± 0.07[b]	2.10 ± 0.26[a]
Pantothenic acid (B5)	0.31 ± 0.02[b]	1.66 ± 0.38[a]
Minerals (mg/100 g)		
Manganese	3.06 ± 0.12[a]	3.93 ± 0.30[a]
Copper	0.28 ± 0.01[a]	0.34 ± 0.04[a]
Potassium	256.0 ± 15.96[a]	319.50 ± 41.83[a]
Phosphorus	204.34 ± 22.61[b]	265.44 ± 14.91[a]
Magnesium	88.24 ± 6.40[a]	114.37 ± 13.65[a]
Iron	2.26 ± 0.04[a]	2.81 ± 0.24[a]
Zinc	1.43 ± 0.06[a]	1.81 ± 0.19[a]
Calcium	29.25 ± 1.67[a]	36.57 ± 4.60[a]
Sodium	1.79 ± 0.05[b]	2.55 ± 0.13[a]
Other (g/100 g)		
Beta-glucan	0.5 ± 0.14[a]	0.31 ± 0.16[a]

The values are the means of duplicate determinations (n = 2) ± standard deviations

Different letters in the same row indicate statistically significant differences ($p < 0.05$)

ND not detected

carbohydrate metabolic processes, which aids in carbohydrate breakdown, as well as enzymes such as isocitrate lyase, which may allow the fungus to metabolize fatty acids and alternative carbon sources during the tricarboxylic acid cycle (Desiderio et al., 2025). Collectively, these enzymes enable fungi to effectively consume and convert carbon sources into protein, sugar, and other metabolites (Kumar et al., 2022; Song et al., 2021). This metabolic efficiency likely explains the significant reduction in carbohydrate content alongside the concurrent increase in protein content observed in the fermented wheat. Nevertheless, specific enzyme activity assays were not performed in the present study. Our results revealed no significant changes in the crude fat, ash, total sugar, or dietary fibre contents of wheat after SSF with the *S. commune*. Contrasting effects were observed in black soybean fermented with mushrooms by Li et al. (2023), who reported that crude fat increased from 18.69% to 22.38% with *Ganoderma oregonense* and to 22.9% with *Coriolus versicolor* and that dietary fibre decreased from 31.70% to 22.90% and 24.29% with *G. oregonense* and *C. versicolor*, respectively. These differences may arise from the considerable variation in the type and catalytic activity of enzymes produced by different mushroom species in response to distinct substrate environments (Pérez-Chávez et al., 2022).

Cereals are good sources of minerals and vitamins, with many studies demonstrating an increased content of B-group vitamins, such as zinc, calcium, magnesium, and iron, and increased availability of minerals in fermented samples (Ganguly et al., 2021). One of the contributing factors to the changes in mineral composition is the reduction in phytic acids and tannins that bind the minerals and the loosening of the substrate's complex matrix that embeds the minerals by the action of phytase and α-amylase during fermentation (Nkhata et al., 2018). As shown in Table 2, fermentation of wheat with *S. commune* appreciably increased the contents of niacin (vitamin B3) and pantothenic acid (vitamin B5) up to 2.7-fold and 5.4-fold, respectively. In addition, a previous study reported a decrease in the β-glucan content caused by fermentation, as reported in barley and oats (Jurkaninová et al., 2024). The insignificant change in β-glucan, a type of dietary fibre, and the total dietary fibre content between unfermented wheat and *S. commune*-fermented wheat suggests an equilibrium between fibre degradation and synthesis during SSF. This outcome is hypothesized to result from two simultaneous, opposing enzymatic actions: the hydrolysis of wheat β-glucans and the synthesis of mushroom β-glucans. *Schizophyllum commune* may release glucan 1,3-β-glucosidase (Desiderio et al., 2025), which may degrade wheat β-glucans by hydrolysing their β−1,3 linkages. This reduction is counterbalanced by the active mycelial synthesis of mushrooms' own β-glucans, which is catalyzed by enzymes such as 1,3-β-glucan synthases (Yang et al., 2023).

Extraction yield of unfermented and *S. commune*-fermented wheat

A comparative analysis of various solvents is essential to determine the most effective system for recovering bioactive compounds. Owing to the diverse solubilities and polarities of these compounds, a multi-solvent approach is often necessary. In the present study, water was prioritized as a primary extraction solvent due to its environmental safety and non-toxic nature regarding human cells (Mihaylova & Lante, 2019). While hot-water extraction is commonly employed to solubilize the chitinous mycelial cell walls and extract polysaccharides and β-glucans (Bleha et al., 2022), high temperatures may degrade certain thermolabile bioactive components. Consequently, cold-water extraction was also performed to preserve these sensitive compounds. Furthermore, ethanol was selected for its high extraction efficiency, while ethyl acetate was utilized

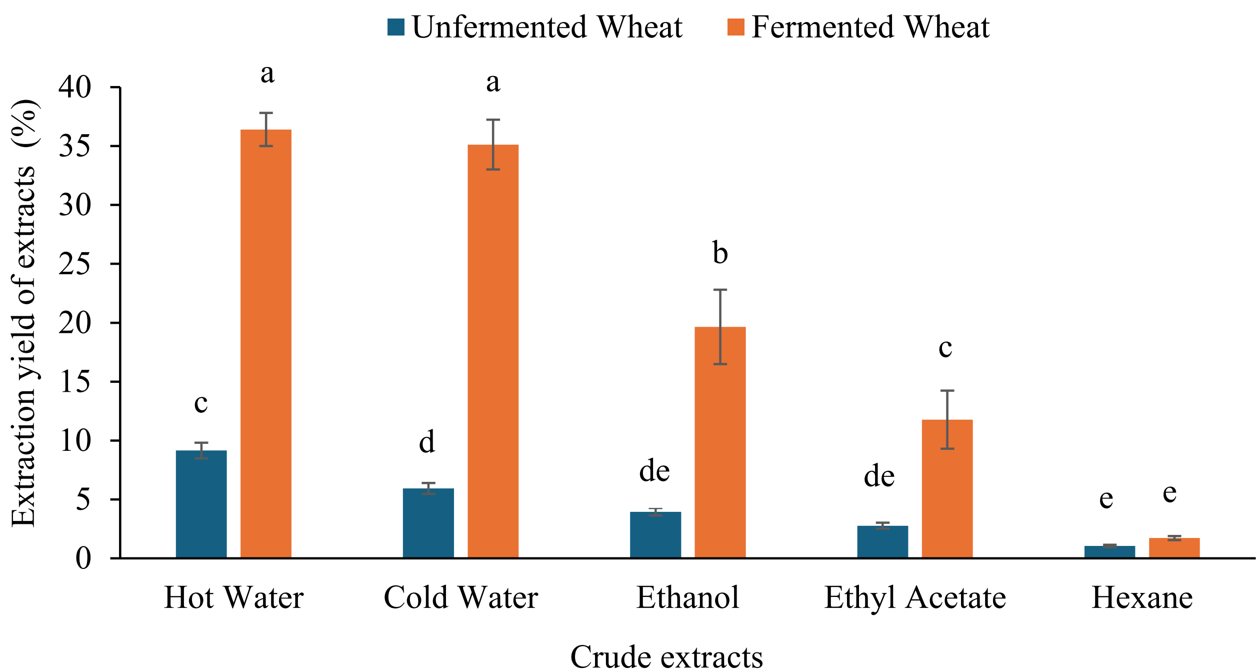


Fig. 1 Extraction yield percentage of unfermented wheat and *S. commune*-fermented wheat crude extracts. Different letters across all bars indicate statistically significant differences ($p< 0.05$)

for its ability to recover a broad range of both polar and non-polar metabolites (Barthwal & Mahar, 2024).

The extraction yields of unfermented and fermented wheat grains are depicted in Fig. 1. Fermented wheat produced higher yields than its unfermented counterpart, which aligns with previous findings by Subramaniam et al. (2014) regarding wheat fermented with *Ganoderma* species. In their study, hot-water extract yields increased from 0.51 g/100 g in unfermented wheat to 1.45 g/100 g (*G. australe*), 1.39 g/100 g (*G. neojaponicum*), and 1.73 g/100 g (*G. lucidum*). In the present study, the highest extraction yield for fermented wheat was obtained through hotwater extraction (36.41%), followed by the cold-water extraction (35.13%), with the lowest yield recovered from hexane (1.72%). These results underscore the synergistic impact of fermentation and solvent polarity on yield, demonstrating that polar solvents are significantly more effective at maximizing the recovery of fermented wheat components. Consistent with these observations, Zhang et al. (2012) demonstrated a direct correlation between solvent polarity and extraction yield in wheat fermented with *Cordyceps militaris*, where the highest yields were obtained using acidified water, followed by 70% ethanol and 70% acetone.

Table 3 Total phenolic and total flavonoid contents of unfermented and *S. commune*-fermented wheat crude extracts

Sample	Total phenolic content (µg GAE/mg extract)	Total flavonoid content (µg QE/mg extract)
Unfermented wheat		
Hot water	4.96 ± 0.16^{e}	0.81 ± 0.02^{d}
Cold water	6.89 ± 0.09^{d}	1.02 ± 0.05^{bc}
Ethanol	4.78 ± 0.13^{e}	0.95 ± 0.04^{c}
Ethyl acetate	9.88 ± 0.04^{c}	0.99 ± 0.01^{bc}
Hexane	1.17 ± 0.02^{f}	0.54 ± 0.03^{d}
S. commune-fermented wheat		
Hot water	16.89 ± 0.51^{b}	1.46 ± 0.11^{a}
Cold water	18.99 ± 0.36^{a}	1.57 ± 0.13^{a}
Ethanol	9.16 ± 0.65^{c}	1.08 ± 0.02^{b}
Ethyl acetate	6.64 ± 1.39^{d}	0.99 ± 0.02^{bc}
Hexane	1.91 ± 0.06^{f}	0.75 ± 0.04^{d}

Different letters in the same column indicate statistically significant differences ($p < 0.05$)

Polyphenolic components of crude extracts from unfermented and *S. commune*-fermented wheat

Phenolic compounds are plant secondary metabolites found in cereals and exist in various fractions, including free and soluble, conjugated, and non-soluble (bound) forms. These compounds possess many health-promoting properties, such as antioxidative and anti-inflammatory effects. For systemic activity, however, the compounds must be soluble to be absorbed and penetrate the human bloodstream (Tsafrakidou et al., 2020). As described by Dey et al. (2016), SSF can be utilized to increase the total phenolic content and the subsequent antioxidant capacity of solid substrates and improve the bioavailability of phenolic compounds. Ferulic acid, the most abundant phenolic acid in wheat, is strongly bound to indigestible cell wall material, limiting its bioavailability (Călinoiu & Vodnar, 2018). During grain colonization, the enzymatic activity of mushroom mycelia breaks down grain cell walls, altering both the physical and biological properties of the grains. These changes often lead to improved nutrient bioavailability and increased levels of health-promoting compounds, such as phenolics. The metabolic activities of mycelia enhance the extractability and bioavailability of phenolics and flavonoids by releasing bound phenolics into their free form and synthesizing new compounds (Adebo & Medina-Meza, 2020). This increase in total phenolics (TPC) and flavonoids (TFC) has been observed in various fermented samples, including wheat bran (Tanaskovic et al., 2021). As shown in Table 3, the total phenolic and flavonoid contents of wheat were improved upon fermentation with *S. commune*. This increase is ostensibly linked to the action of fungal cell wall-degrading enzymes, such as cellulases and xylanases, which facilitate the liberation of bound phenolic compounds from the wheat matrix during fermentation. Although direct enzymatic quantification was not performed in this study, the elevated concentration of bioactive constituents strongly suggests the presence of robust enzymatic activity by *S. commune*. Similar observations have been documented in previous studies involving the fermentation of wheat with various mushroom species. For instance, Boonthatui et al. (2021) observed a considerable increase in TPC from 0.56 mg GAE/g in unfermented wheat to 8.56 mg GAE/g in *S. commune*-fermented wheat water extract following 8 days of SSF. Similarly, Zhai et al. (2015) reported a 1.58-fold increase in TPC of *Agaricus blazei*-fermented wheat ethanolic extract compared with its unfermented counterpart. Conversely, Subramaniam et al. (2014) noted a significant reduction in TPC in both water and ethanolic extracts of *G. lucidum*-fermented wheat. Specifically, the TPC in the water extract decreased from 27.37 mg GA/g extract to 17.73 mg GA/g extract, while the ethanolic extract showed a reduction from 56.01 mg GA/g extract to 32.87 mg GA/g extract in the fermented samples. The present study revealed that the water extracts of fermented wheat contained considerably higher TPC and TFC than the other extracts. Consistent with this findings, Huang et al. (2011), observed a greater TPC in the hot-water extract of *Grifola frondosa*-fermented wheat compared to its ethanolic counterpart. In this study, the TPC of unfermented wheat extracts ranged from 1.17–9.88 µg GAE/mg, whereas fermented wheat extracts ranged from 1.90–18.99 µg GAE/mg. The cold-water extract exhibited the highest TPC, representing a 2.76-fold increase over its unfermented counterpart. Notably, the highest TPC in the unfermented wheat was found in the ethyl acetate fraction. This suggests that intermediate-polarity solvents are superior for recovering a diverse profile of semipolar phenolics such as certain flavonoids and aglycones, compared to highly polar (water) or non-polar (hexane) solvents (Galanakis et al., 2013). Phenolic compounds in grains such as wheat exist in soluble free forms, soluble conjugates (esterified with sugars or low-molecular-mass compounds), or insoluble forms covalently cross-linked to cell wall components (Santos et al., 2019). The differing polarities of these forms directly govern their extractability. For instance, water primarily targets highly polar soluble conjugates and free forms, whereas hexane recovers lipophilic fraction. However, the extractability of these compounds is also highly influenced by process parameters such as time and temperature. Enzymatic biotransformation during *S. commune* fermentation likely generated smaller, more polar phenolic metabolites, thereby leading to the observed increase in TPC within the water extracts. Overall, fermentation with *S. commune* enhanced the TPC from approximately 0.274 µg GAE/mg in unfermented wheat to 6.672 µg GAE/mg in the fermented substrate.

Flavonoids are key compounds in wheat that contribute to its health benefits. Microbial fermentation significantly alters the flavonoid profile in plant-based materials through processes such as glycosylation, deglycosylation, methylation, and glucuronidation, with specific changes depending on the microbial species used (Hyunh et al., 2014). The total flavonoid content of unfermented wheat extracts ranged from 0.54 to 1.02 µg QE/mg extract, whereas that of *S. commune*-fermented extracts ranged from 0.75 to 1.57 µg QE/mg extract. This represents a 1.1- to 1.8-fold increase in total flavonoid content for most fermented extracts compared with their unfermented controls, except for the ethyl acetate extract, whose total flavonoid content slightly decreased. The extraction yield revealed that the TFC increased from approximately 0.027 µg QE/mg unfermented wheat to 0.552 µg QE/mg fermented wheat.

Antioxidative properties of crude extracts from unfermented and *S. commune*-fermented wheat

Assessing the antioxidative properties of edible materials and food products is essential not only for ensuring quality but also for gauging their efficacy in preventing and managing diseases associated with oxidative stress, as well as maintaining overall health. In the present study,

Table 4 Antioxidative activities of unfermented and *S. commune*-fermented wheat crude extracts

Sample	DPPH scavenging (%)	β-carotene bleaching inhibition (%)	Ferric reducing (μM FE/mg extract)
Unfermented wheat			
Hot water	ND	40.44 ± 3.18[ab]	165.61 ± 6.64[e]
Cold water	ND	59.07 ± 3.61[a]	272.22 ± 5.04[c]
Ethanol	19.67 ± 0.83[d]	38.48 ± 9.95[c]	45.82 ± 4.63[g]
Ethyl acetate	39.29 ± 2.38[b]	13.36 ± 2.62[d]	20.63 ± 7.14[h]
Hexane	12.13 ± 0.28[e]	18.87 ± 5.91[d]	11.74 ± 5.59[h]
S. commune-fermented wheat			
Hot water	40.92 ± 1.48[b]	55.27 ± 1.49[bc]	395.44 ± 10.00[a]
Cold water	51.65 ± 5.91[a]	64.95 ± 0.21[a]	312.11 ± 13.10[b]
Ethanol	20.70 ± 2.24[cd]	63.73 ± 2.81[a]	191.37 ± 9.58[d]
Ethyl acetate	25.65 ± 3.58[c]	26.23 ± 4.02[cd]	129.52 ± 2.31[f]
Hexane	15.38 ± 0.42[de]	36.89 ± 5.91[c]	21.37 ± 6.12[h]
Ascorbic acid	92.44 ± 1.65 (at 0.1 mg/ml)	41.62 ± 3.41 (at 1 mg/mL)	-
Trolox	67.69 ± 1.37 (at 0.1 mg/mL)	90.32 ± 0.85 (at 1 mg/mL)	-

Different letters in the same column indicate statistically significant differences ($p < 0.05$)

ND not detected at the concentration tested

Trolox (a lipophilic vitamin E analogue) and ascorbic acid (a hydrophilic compound) were employed as positive controls in the DPPH scavenging and β-carotene bleaching inhibition assays. These standards were utilized to effectively benchmark activity across the entire polarity spectrum of the extracts, which ranged from highly polar water extracts to non-polar hexane extracts. Additionally, to examine the relationship between the bioactive constituents and functional properties, a Pearson correlation analysis was conducted for TPC and TFC against all antioxidative assays performed in the study (Table 7, additional file 1).

The improved antioxidative effects in fermented plants have been attributed to changes in the value and composition of phytochemicals such as phenolics, amino acids, peptides, and polysaccharides (Chen et al., 2021; Melini & Melini., 2021). However, Dey and Kuhad (2014) reported that the formation of antioxidant compounds during SSF involves a complex biochemical process that has not yet been fully elucidated. As displayed in Table 4, the fermentation of wheat with *S. commune* considerably improved the antioxidant capacity of wheat. Several studies have reported enhancements in the antioxidative properties of wheat upon fermentation, for example, with *Ganoderma* spp. (Subramaniam et al., 2014), *Taiwanofungus salmoneus* (Chiang et al., 2015), *Agaricus blazei* (Zhai et al., 2015), and *S. commune* (Boonthatui et al., 2021). The increase in antioxidative activity is likely due to biochemical changes in wheat during fermentation, where antioxidant compounds, such as phenolics, are released or modified to increase bioavailability (Zhao et al., 2021). The shift in higher antioxidative activities post-fermentation, where the aqueous extract became the most effective solvent, can be attributed to the β-glucosidase activity of *S. commune* (Tovar-Herrera et al., 2018). This enzymatic action likely hydrolyses naturally occurring glycosides (polar sugar-bound phenolics) into more polar, aglycone forms, thus making them more readily extractable into a more polar phase (aqueous and ethanol) and potentially enhancing their intrinsic antioxidative ability (Šelo et al., 2024). In addition to β-glucosidase, other mushroom enzymes, such as laccase, may contribute to the liberation and bio-transformation of phenolic compounds in fermented wheat. This process potentially alters the molecular structure of these metabolites, thereby enhancing the antioxidative potency of the extractable compounds (McCue et al., 2004).

Compared with the organic solvent extracts, the water extracts of the fermented samples presented higher DPPH radical scavenging activities. However, the corresponding water extracts from the unfermented sample showed no detectable scavenging activity. It can be speculated that fermentation with *S. commune* increased the concentration of polar compounds possessing DPPH scavenging potential, which are highly extractable by water. Interestingly, while the compounds extracted by moderately polar ethyl acetate from unfermented wheat possessed good DPPH radical scavenging activities, fermentation with *S. commune* altered the potency of this effect. This observation aligns with the high TPC measured in the unfermented ethyl acetate extract (Table 3), suggesting that the phenolic compounds extracted by ethyl acetate from unfermented wheat may possess stronger DPPH radical scavenging activity than those recovered after fermentation. In a related study, wheat fermented with *A. blazei* presented superior DPPH scavenging activity compared to the unfermented control (Zhai et al., 2015). In the present study, fermentation with *S. commune* significantly ($p < 0.05$) enhanced the ferric-reducing antioxidant activity of the wheat extracts, with improvements ranging from 1.14- to 6.28-fold. While the greatest relative improvement was observed in the ethyl acetate extract, the hot-water extract exhibited the highest overall ferric-reducing capacity. Similar observations regarding the enhanced capacity of water extracts were reported by Boonthatui et al. (2021), who noted a 2.1-fold improvement in the TFC alongside substantially increased antioxidative properties in *S. commune*-fermented wheat after an 8-day fermentation period, including a 10.1-fold increase in DPPH radical scavenging and a 17.3-fold improvement in ferric-reducing capacity. The discrepancy in the magnitude of improvement between studies may be ascribed to the variations in the specific *S. commune* strain, sample drying and extraction procedures, and fermentation parameters. Another study reported a greater increase in the ferric-reducing capacity of an ethanolic extract from wheat fermented with *G. neojaponicum* and a reduction in the ferric-reducing capacity of water and ethanolic extracts from wheat fermented with *G. australe* and *G. lucidum* (Subramaniam et al., 2014). Additionally, the DPPH scavenging and ferric-reducing activities were strongly correlated with the TPC, with values of $r = 0.8244$ and $r = 0.7870$, respectively (additional file 1).

β-Carotene bleaching inhibition activity is a spectrophotometric technique that measures a substance's ability to stop the breakdown or bleaching of β-carotene, a pigment whose orange colour is lost by peroxyl and free radicals. The ability of a drug to scavenge free radicals and prevent lipid peroxidation is shown by a higher percentage of inhibition, which also signifies better antioxidant activity (Dawidowicz & Olszowy, 2015). In the present study, the significantly higher activity of the lipid-soluble Trolox compared to the water-soluble ascorbic acid is consistent with the chemical behaviour of the β-carotene-linoleic emulsion system. This outcome is anticipated, as Trolox partitions more effectively into the hydrophobic lipid phase where the β-carotene resides, thereby offering superior protection against oxidation. Extracts of the fermented wheat significantly ($p < 0.05$) inhibited bleaching to varying degrees. The results showed that extracts from wheat, particularly polar extracts, moderately inhibited β-carotene bleaching, and this effect was enhanced through fermentation with *S. commune*. An increase ranging from 1.1–1.96-fold was observed, with the ethyl acetate extract from fermented wheat showing the greatest improvement. The cold-water and ethanolic extracts from fermented wheat exhibited the strongest inhibitory effects on β-carotene bleaching. However, fermentation with *S. commune* did not result in a significant improvement in the inhibitory activity of the cold-water extract compared to its unfermented counterpart. The bleaching inhibition of the samples was moderately correlated with their TPC ($r = 0.5589$).

Table 5 Anti-inflammatory activities of unfermented and *S. commune*-fermented wheat crude extracts

Sample	Nitric oxide scavenging (%)	Protein denaturation inhibition (%)
Unfermented wheat		
Hot water	40.77 ± 2.05^{bc}	11.92 ± 2.11^{e}
Cold water	41.44 ± 3.37^{bc}	20.57 ± 1.14^{cd}
Ethanol	26.60 ± 3.58^{de}	23.11 ± 0.56^{cd}
Ethyl acetate	21.72 ± 1.22^{ef}	22.20 ± 1.84^{cd}
Hexane	ND	-13.31 ± 5.23^{f}
S. commune-fermented wheat		
Hot water	47.09 ± 2.11^{ab}	30.59 ± 0.48^{c}
Cold water	55.14 ± 2.84^{a}	44.62 ± 3.95^{b}
Ethanol	34.96 ± 4.55^{cd}	58.54 ± 7.05^{a}
Ethyl acetate	26.36 ± 1.22^{de}	30.04 ± 0.63^{c}
Hexane	14.63 ± 3.48^{f}	ND
Gallic acid (at 1 mg/mL)	57.14 ± 2.11	-
Diclofenac sodium (at 1 mg/mL)	-	75.64 ± 2.52

Different letters in the same column indicate statistically significant differences ($p < 0.05$)

ND not detected at the concentration tested

Cuidad-Mulero et al. (2020) reported significant differences in the capacity of methanolic extracts from refined and whole-grain wheat flour to inhibit β-carotene bleaching. To the best of our knowledge, there are no reports on the β-carotene bleaching-inhibiting activity of fermented wheat or any other fermented grain. A previous study demonstrated a decrease in the antioxidative properties of grains fermented with mushrooms, for example, a reduction in $ABTS^{+}$ radical scavenging activity in the methanol extract of buckwheat fermented with *S. sanghuang* (Song et al., 2021). The aqueous extracts of wheat fermented with *G. australe* had lower antioxidative effects than unfermented wheat, which was highly correlated with their respective TPC (Subramaniam et al., 2014). The authors also observed distinct variations in the antioxidative properties of unfermented and fermented wheat extracts, which were contingent upon the specific *Ganoderma* species and extraction solvent employed.

Anti-inflammatory properties of crude extracts from unfermented and fermented wheat

Inflammation is fundamentally linked to the pathogenesis of diseases such as cancer, diabetes, obesity, arthritis, and cardiovascular diseases. Nitric oxide (NO) is a crucial physiological mediator of numerous processes, such as T-cell activation (Andrabi et al., 2023). However, its excessive endogenous production plays a role in the development of certain chronic inflammatory conditions (Kim & Lee, 2025). In this study, the anti-inflammatory potential of *S. commune*-fermented wheat was evaluated based on the capacity of its extracts to scavenge NO. The NO scavenging activities of the various unfermented and fermented extracts are summarized in Table 5. Additionally, to examine the relationship between the bioactive compounds and functional properties, a Pearson correlation analysis was performed for the TPC and TFC against all anti-inflammatory assays conducted in this study (Table 7, additional file 1).

Fermentation with *S. commune* demonstrably impacted the NO scavenging ability of the wheat extracts. Compared to their respective unfermented counterparts, the fermented extracts exhibited an increase in activity ranging from 1.15- to 1.33-fold. The only exception was the hexane extract, which showed undetectable scavenging activity in the unfermented state but reached 14.63% after fermentation. A strong correlation between the TPC and TFC and NO-scavenging activity ($r = 0.7931$ and $r = 0.8469$, respectively) was also observed. The ethanol extract derived from *T. salmoneus*-fermented wheat exhibited concentration-dependent inhibition of NO production, ranging from 16.60% to 41.02% at 50–500 µg/mL, when tested in lipopolysaccharide (LPS)-induced RAW264.7 macrophages (Chiang et al., 2015). Xu et al. (2023) reported that polysaccharides from *G. frondosa*-fermented wheat significantly promoted NO synthesis in uninduced RAW 264.7 cells, increasing NO production up to 2.76 µm, representing a 3.88-fold increase.

Protein denaturation is a biological process that occurs during a chronic inflammatory response, impairing tissue functions and subsequently leading to various inflammatory diseases (Nehru et al., 2023; Zhang et al., 2019). Therefore, the ability of a substance to hinder protein denaturation indicates its potential for anti-inflammatory effects (Hasan et al., 2023). As displayed in Table 5, fermentation with *S. commune* successfully improved the capacity of wheat to inhibit protein denaturation. The fermented wheat extracts significantly ($p < 0.05$) inhibited protein denaturation to varying degrees, with the crude ethanolic extract providing the highest level of BSA protection against heat-induced denaturation. Interestingly, the unfermented hexane extract exerted a pro-inflammatory effect by increasing the rate of protein denaturation. A similar trend was observed by Shabbir et al. (2022), who reported that ethanolic extracts from black soybean fermented with *Pediococcus acidilactici* exhibited greater inhibitory activity than their unfermented counterparts. While the anti-inflammatory properties of various fermented wheat products have been documented, to the best of our knowledge, this is the first study to report the protein denaturation inhibitory activity of wheat fermented with *S. commune*.

Chemical composition of the most active extract from *S. commune*-fermented wheat

For the LC–QTOF MS study, the cold-water extract was selected because it exhibited the highest antioxidative and anti-inflammatory activities, as detailed in Tables 4 and 5. The chemical composition of the cold-water extract was profiled via LC-QTOF MS in untargeted mode to quantify the metabolite signals. A total of 70 distinct metabolites were putatively identified (Table 8, additional file 2), exhibiting a mass inaccuracy of molecular ions within 5 ppm. Metabolite identification in this untargeted analysis is classified as Level 2 confidence according to the Metabolomics Standards Initiative (MSI) guidelines (Salek et al., 2013), as assignments were made by comparing mass accuracy, parent ion isotope distribution ratios, and retention times (RTs) with existing references, without confirmation using external pure standards. Table 6 details the key compounds identified in the extract, including the most intense metabolic peaks as well as less abundant compounds known for their significant nutritional and health-promoting properties. The results suggest that the health-promoting properties of cold-water extracts could be attributed to the presence of many notable compounds known for their antioxidative and/or anti-inflammatory properties. A study by Shabbir et al. (2022) suggested that bioactive compounds such as daidzein, (+)-catechin, rutin, and p-coumaric acid are the contributing factors to the anti-inflammatory effects of black soybean fermented by *P. acidilactici.* The presence of gallic acid, a known antioxidant with free-radical scavenging and lipid peroxidation protective effects (Hadidi et al., 2024), likely contributes to the observed DPPH and nitric oxide scavenging activities as well as the β-carotene bleaching inhibition activity of the cold-water extract. Phenolic acids such as rosmarinic, vanillic and ferulic acids have been reported to potently inhibit β-carotene

Table 6 LC-QTOF MS analysis of nutritional and health-promoting compounds in *S. commune*-fermented wheat extract

Classification	Proposed compound	Molecular formula	Molecular weight	Observed m/z	Mass error (ppm)	RT (min)	Reported bioactivities (references)
Carbohydrate	α-Kojibiose	$C_{12}H_{22}O_{11}$	342.116	341.109	−1.1	0.63	Prebiotic (Ahmed et al., 2022)
	Galactose	$C_6H_{12}O_6$	180.063	179.056	−3.5	0.62	Essential carbohydrate (Conte et al., 2021)
	Isomaltose	$C_{12}H_{22}O_{11}$	342.116	341.109	−1.1	2.26	Prebiotic (Tiangpook et al., 2023)
Coumarin	Calycanthoside	$C_{17}H_{20}O_{10}$	384.107	383.099	3.6	0.58	Antioxidative, anti-inflammatory (Al-Naimi et al., 2025)
	Fraxin	$C_{16}H_{18}O_{10}$	370.090	369.083	0.7	0.58	Antioxidative, Anti-inflammatory (Patel & Patel, 2023)
Flavonoid	3-O-β-D-Galactopyranosyl quercetin	$C_{21}H_{22}O_{12}$	466.111	465.104	0.6	8.05	Not explicitly described to date
	Kaempferol-3-O-β-D-glucopyranoside	$C_{21}H_{20}O_{11}$	448.101	447.093	0	10.3	Antioxidative, anti-inflammatory (Chen et al., 2023)
	6-Hydroxykaempferol-3-O-glucoside	$C_{21}H_{20}O_{12}$	464.095	463.088	−0.6	8.92	Not explicitly described to date
Organic Acid	Glucuronic acid	$C_6H_{10}O_7$	194.042	193.035	−2.4	0.62	Antioxidative (Chou et al., 2024)
	Citric acid	$C_6H_8O_7$	192.026	191.019	−2.9	0.98	Antioxidative, anti-inflammatory (Singh et al., 2022)
	Quinic acid	$C_7H_{12}O_6$	192.063	191.056	−3.1	0.67	Antioxidative (Benali et al., 2022)
Phenols	Undulatoside A	$C_{16}H_{18}O_9$	354.096	353.089	2.9	0.58	Anti-inflammatory (Peng et al., 2015)
	Cistanoside F	$C_{21}H_{28}O_{13}$	488.153	487.146	−0.1	8.53	Antioxidative, anti-inflammatory (Ma et al., 2025)
Phenolic Acid	Gallic acid	$C_7H_6O_5$	170.021	169.014	−3.2	1.98	Antioxidative, anti-inflammatory (Hadidi et al., 2024)
	3-Ethoxy-4,5-dihydroxybenzoic acid	$C_9H_{10}O_5$	198.052	197.045	−2.6	7.34	Not explicitly described to date
Tannin	3,6-Di-O-Galloyl-β-D-glucose	$C_{20}H_{20}O_{14}$	484.085	483.078	0	3.43	Not explicitly described to date
	1-Galloyl-β-D-glucose	$C_{13}H_{16}O_{10}$	332.074	331.067	−0.8	1.79	Antioxidative, Anti-inflammatory Khan et al. (2022)
	Castalagin	$C_{41}H_{26}O_{26}$	934.073	933.065	1.4	3.86	Anti-inflammatory (Piazza et al., 2024)
	Corilagin	$C_{27}H_{22}O_{18}$	634.081	633.074	1.2	2.20	Antioxidative, Anti-inflammatory (Widowati et al., 2021)
Xanthone	Neomangiferin	$C_{25}H_{28}O_{16}$	584.136	583.129	−2.6	0.56	Antioxidative, anti-inflammatory (López-Cárdenas et al., 2023)

bleaching (Sevgi et al., 2015). The flavonoid kaempferol-3-O-β-D-glucopyranoside, known as astragalin, is also a potential major contributor to the antioxidative effects of the extract. This finding is supported by previous research showing that it has ferric-reducing and DPPH inhibitory effects (Li et al., 2017). Other putative compounds in the extract, such as calycanthoside (isofraxidin-7-glucoside) and cistanoside F, possess documented antioxidative and anti-inflammatory properties (Al-Naimi et al., 2025; Ma et al., 2025). Additionally, 1-galloyl-β-D-glucose, commonly referred to as β-glucogallin, is a hydrolyzable tannin with multifaceted health-promoting properties (Khan et al., 2022) and may also likely contributes to the extract's antioxidative and anti-inflammatory activities.

Quinic acid and glucuronic acid, which are organic acids, have been reported to possess many health-promoting properties (Benali et al., 2022; Chou et al., 2024). Interestingly, Hu et al. (2016) reported weak antioxidative activities of glucuronic acid based on FRAP, DPPH-radical scavenging, and β-carotene bleaching inhibition assays. Conversely, a study by Deng et al. (2023) suggested that glucuronic acid could be the key compound that enhances the antioxidative and anti-inflammatory properties of polysaccharides derived from the *Tremella fuciformis* mushroom. Citric acid, also putatively identified in the extract, is widely recognized for its nutritional and health-promoting properties. As citric acid is associated with antioxidant and anti-inflammatory activity at the cellular level (Singh et al., 2022), its presence is largely attributable to the overall bioactivity of the cold-water extracts. Furthermore, its ability to improve the bioavailability of essential minerals such as iron (Jaiswal & Lakshmi, 2022) could further increase the nutritional value of *S. commune*-fermented wheat, positioning it as a more effective functional food. Additionally, the putative identification of isomaltose, a key component of the prebiotic isomaltooligosaccharides (IMOs) (Tiangpook et al., 2023), suggests that *S. commune*-fermented wheat may possess prebiotic potential, further enhancing its value as a functional ingredient.

Overall, the nutritional shifts and enhanced bioactivity observed in *S. commune*-fermented wheat may be attributed to various factors inherent in the complex fermentation process. Depending on the type of grain, species of microorganism involved, fermentation parameters, and extraction process, many types of compounds are produced and released, which may exhibit health-promoting properties. Our collective results indicate that higher solvent polarity facilitated the recovery of more potent bioactive components. Specifically, *S. commune*-fermented wheat yielded the most effective compounds in the water extract, which exhibited superior antioxidative and anti-inflammatory activities compared to extracts obtained with non-polar solvents. However, given the limitations of this study, utilizing solvent mixtures and optimizing extraction parameters may further improve the recovery efficiency of these functional metabolites. Additionally, the observed potent antioxidative and anti-inflammatory activities of the cold-water extract are likely not attributable to a single constituent. Instead, they may result from the synergistic effect of a unique combination of bioactive compounds putatively identified via LC–MS, which together demonstrate greater potency than individual compounds alone. While the current untargeted approach provides a comprehensive metabolic profile, future studies should focus on the targeted quantification of key metabolites, such as gallic acid and astragalin, using analytical standards to fully confirm their concentrations and specific biological contributions.

Conclusions

Exploring the potential of mushroom mycelia is critical in the contemporary era, where significant focus is placed on developing sustainable, ready-to-consume food products. As the production of mushroom-fermented wheat is a standard practice in the cultivation industry, *S. commune* mycelia and its fermented substrate warrant further exploration as a novel functional food base. Given the enhanced nutritional profile and improved bioactive properties of the whole fermented material, specific applications for *S. commune*-fermented wheat may include its use as a functional flour fortifier in baked goods, high-protein or high-fibre base for snack bars, or an ingredient in powdered meal replacements and nutritional supplements. Furthermore, the use of a simple SSF method involving minimal ingredients and requiring only basic equipment with readily available grains such as wheat inherently offers high scalability and cost-effectiveness for industrial-scale production. This positions the *S. commune*-fermented wheat products as a viable ingredient for the functional food market. Future research should focus on isolating the key compounds and elucidating their precise mechanisms of action, utilizing in vitro cellular models and subsequent in vivo studies using animal models to fully validate and leverage the health benefits of this sustainable resource. Such research could establish a greater role for *S. commune* and other mushroom-fermented substrates in the functional food industry, addressing the consumer demand for products that combine convenience with therapeutic value.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-026-00367-y.

Additional file 1: Table 7 Pearson correlation analysis. Full list of correlation values and significant values between TPC and TFC and the antioxidative and anti-inflammatory properties of *S. commune*-fermented wheat extracts.

Additional file 2: Table 8 LC–QTOF MS analysis of compounds in *S. commune*-fermented wheat extract. Full list of putative compounds in the cold-water extract of *S. commune*-fermented wheat identified through LC-QTOF MS.

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Authors' contributions

D.L.A.R: writing – original draft, methodology, investigation, formal analysis, conceptualization, visualization, A.I: conceptualization, supervision, validation, funding acquisition, writing – review & editing, N.A.B: supervision, writing – review & editing. N.K.K: supervision, M.I.M.L. & K.A.K: methodology, investigation, writing – original draft.

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Data availability
All data supporting the findings of this study are available within the paper and its supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Dr. Aimin Ismail is editorial board member of *Food Production, Processing and Nutrition*, and he was not involved in the review or decision related to this manuscript.

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References

Abd Ghafar, S. Z., Mediani, A., Maulidiani, Ramli, & N.S., & Abas, F. (2018). Antioxidant, α-glucosidase, and nitric oxide inhibitory activities of *Phyllanthus acidus* and LC–MS/MS profile of the active extract. *Food Bioscience, 25*, 134–140. https://doi.org/10.1016/j.fbio.2018.08.009

Abd Razak, D. L., Jamaluddin, A., Abd Rashid, N. Y., Abd Ghani, A., & Abdul Manan, M. (2019). Assessment of fermented broken rice extracts for their potential as functional ingredients in cosmeceutical products. *Annals of Agricultural Sciences, 64*(2), 176–182. https://doi.org/10.1016/j.aoas.2019.11.003

Abd Razak, D. L., Jamaluddin, A., Abd Rashid, N. Y., Sani, N. A., & Abdul Manan, M. (2020). Assessment of cosmeceutical potentials of selected mushroom fruitbody extracts through evaluation of antioxidant, anti-hyaluronidase and anti-tyrosinase activity. *J, 3*, 329–342. https://doi.org/10.3390/j3030026

Adebo, O. A., & Medina-Meza, I. G. (2020). Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. *Molecules, 25*, 927. https://doi.org/10.3390/molecules25040927

Ahmed, A., Khan, T. A., Dan Ramdath, D., Kendall, C. W. C., & Sievenpiper, J. L. (2022). Rare sugars and their health effects in humans: A systematic review and narrative synthesis of the evidence from human trials. *Nutrition Reviews, 80*(2), 255–270. https://doi.org/10.1093/nutrit/nuab012

Al-Naimi, M. S., Abu-Raghif, A. R., Mansoor, A. F. A., & Fawzi, H. A. (2025). Isofraxidin attenuates lipopolysaccharide-induced cytokine release in mice lung and liver tissues by inhibiting inflammation and oxidative stress. *Biomedicines, 13*(3), 653. https://doi.org/10.3390/biomedicines13030653

Andrabi, S. M., Sharma, N. S., Karan, A., Shahriar, S. M. S., Cordon, B., Ma, B., & Xie, J. (2023). Nitric oxide: Physiological functions, delivery, and biomedical applications. *Advanced Science, 10*(30), Article 2303259. https://doi.org/10.1002/advs.202303259

Antunes, F., Marçal, S., Taofiq, O., Morais, A. M. M. B., Freitas, A. C., Ferreira, I. C. F. R., & Pintado, M. (2020). Valorization of mushroom byproducts as a source of value-added compounds and potential applications. *Molecules, 25*, Article 2672. https://doi.org/10.3390/molecules25112672

Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S. S. (2016). Role of antioxidants and natural products in inflammation. *Oxidative Medicine and Cellular Longevity, 2016*, Article 5276130. https://doi.org/10.1155/2016/5276130

Barthwal, R., & Mahar, R. (2024). Exploring the significance, extraction, and characterization of plant-derived secondary metabolites in complex mixtures. *Metabolites, 14*(2), Article 119. https://doi.org/10.3390/metabo14020119

Benali, T., Bakrim, S., Ghchime, R., Benkhaira, N., El Omari, N., Balahbib, A., Taha, D., Zengin, G., Hasan, M. M., Bibi, S., & Bouyahya, A. (2022). Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnology and Genetic Engineering Reviews, 40*(4), 3408–3437. https://doi.org/10.1080/02648725.2022.2122303

Benson, K. F., Stamets, P., Davis, R., Nally, R., Taylor, A., Slater, S., & Jensen, G. S. (2019). The mycelium of the *Trametes versicolor* (Turkey tail) mushroom and its fermented substrate each show potent and complementary immune activating properties *in vitro*. *BMC Complementary and Alternative Medicine, 19*, Article 342. https://doi.org/10.1186/s12906-019-2681-7

Bleha, R., Třešnáková, L., Sushytskyi, L., Capek, P., Čopíková, J., Klouček, P., Jablonský, I., & Synytsya, A. (2022). Polysaccharides from basidiocarps of the polypore fungus *Ganoderma resinaceum*: Isolation and structure. *Polymers, 14*(2), Article 255. https://doi.org/10.3390/polym14020255

Bobo-García, G., Davidov-Pardo, G., Arroqui, C., Vírseda, P., Marín-Arroyo, M. R., & Navarro, M. (2015). Intralaboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *Journal of the Science and Food Agriculture, 95*(1), 204–209. https://doi.org/10.1002/jsfa.6706

Boonthatui, Y., Chongsuwat, R., & Kittisakulnam, S. (2021). Production of antioxidant bioactive compounds during mycelial growth of *Schizophyllum commune* on different cereal media. *Chiang Mai University Journal of Natural Sciences, 20*(2), e2021032. https://doi.org/10.12982/CMUJNS.2021.032

Călinoiu, L. F., & Vodnar, D. C. (2018). Whole grains and phenolic acids: A review on bioactivity, functionality, health benefits and bioavailability. *Nutrients, 10*(11), 1615. https://doi.org/10.3390/2Fnu10111615

Cantato, A. G., & Conte-Junior, A. A. (2025). Mushrooms in innovative food products: Challenges and potential opportunities as meat substitutes, snacks and functional beverages. *Trends in Food Science & Technology, 156*, 104868. https://doi.org/10.1016/j.tifs.2025.104868

Chen, Q., Wang, R., Wang, Y., An, X., Liu, N., Song, M., Yang, Y., Yin, N., & Qi, J. (2021). Characterization and antioxidant activity of wheat bran polysaccharides modified by *Saccharomyces cerevisiae* and *Bacillus subtilis* fermentation. *Journal of Cereal Science, 97*, Article 103157. https://doi.org/10.1016/j.jcs.2020.103157

Chen, J., Zhong, K., Qin, S., Jing, Y., Liu, S., Li, D., & Peng, C. (2023). Astragalin: A food-origin flavonoid with therapeutic effect for multiple diseases. *Frontiers in Pharmacology, 14*, Article 1265960. https://doi.org/10.3389/fphar.2023.1265960

Chiang, S. S., Ulziijargal, E., Chien, R. C., & Mau, J. L. (2015). Antioxidant and anti-inflammatory properties of solid-state fermented products from a medicinal mushroom, *Taiwanofungus salmoneus* (higher Basidiomycetes) from Taiwan. *International Journal of Medicinal Mushrooms, 17*(1), 21–32. https://doi.org/10.1615/intjmedmushrooms.v17.i1.30

Chou, Y.-C., Lin, H.-W., Wang, C.-Y., Hsieh, C.-C., Santoso, S. P., Lin, S.-P., & Cheng, K.-C. (2024). Enhancing antioxidant benefits of kombucha through optimized glucuronic acid by selected symbiotic fermentation culture. *Antioxidants, 13*(11), Article 1323. https://doi.org/10.3390/antiox13111323

Ciudad-Mulero, M., Barros, L., Fernandes, Â., Ferreira, C. F. R., & I., Callejo, M.J., Matallana-González, M.C., Fernández-Ruiz, V., Morales, P., & Carrillo, J.M. (2020). Potential health claims of durum and bread wheat flours as functional ingredients. *Nutrients, 12*(2), 504. https://doi.org/10.3390/nu12020504

Conte, F., van Buuringen, N., Voermans, N. C., & Lefeber, D. J. (2021). Galactose in human metabolism, glycosylation and congenital metabolic diseases: Time for a closer look. *Biochimica Et Biophysica Acta, 1865*(8), Article 129898. https://doi.org/10.1016/j.bbagen.2021.129898

Cui, L., Li, D., & Liu, C. (2012). Effect of fermentation on the nutritive value of maize. *International Journal of Food Science and TechnoloGy, 47*, 755–760. https://doi.org/10.1111/j.1365-2621.2011.02904.x

Dawidowicz, A. L., & Olszowy, M. (2015). Depletion/protection of β-carotene in estimating antioxidant activity by β-carotene bleaching assay. *Journal of Food Science and Technology, 52*, 7321–7328. https://doi.org/10.1007/s13197-015-1820-5
De Cianni, R., Pippinati, L., & Mancuso, T. (2023). A systematic review on drivers influencing consumption of edible mushrooms and innovative mushroom-containing products. *Appetite, 182*, 106454. https://doi.org/10.1016/j.appet.2023.106454
Debnath, S., Bhattacharya, S., Das, P., & Saha, A. K. (2020). Cultivation of a wild strain of *Schizophyllum commune* on agro-industrial wastes. *Kavaka, 55*, 77–83. https://doi.org/10.36460/Kavaka/55/2020/77-83
Deng, W., Wu, L., Xiao, Z., Li, Y., Zheng, Z., & Chen, S. (2023). Structural characterization and anti-inflammatory activity of polysaccharides from *Tremella fuciformis* on monosodium urate-stimulated RAW264.7 macrophages. *Foods, 12*(24), Article 4398. https://doi.org/10.3390/foods12244398
Desiderio, A., Goppa, L., Santambrogio, C., Brocca, S., Buratti, S., Girometta, C. E., Sarkar, M., Venuti, M. T., Savino, E., Rossi, P., & Ferrari, E. (2025). Improving the proteome-mining of *Schizophyllum commune* to enhance medicinal mushroom applications. *Journal of Fungi, 11*(2), Article 120. https://doi.org/10.3390/jof11020120
Dey, T. B., & Kuhad, R. C. (2014). Upgrading the antioxidant potential of cereals by their fungal fermentation under solid-state cultivation conditions. *Letters in Applied Microbiology, 59*(5), 493–499. https://doi.org/10.1111/lam.12300
Dey, T. B., Chakraborty, S., Jain, K. K., Sharma, A., & Kuhad, R. C. (2016). Antioxidant phenolics and their microbial production by submerged and solid-state fermentation process: A review. *Trends in Food Science & Technology, 53*, 60–74. https://doi.org/10.1016/j.tifs.2016.04.007
Espinosa-Paez, E., Alanis-Guzman, M. G., Hernandez-Luna, C. E., Baez-Gonzalez, J. G., Amaya-Guerra, C. A., & Andreas-Grau, A. M. (2017). Increasing antioxidant activity and protein digestibility in *Phaseolus vulgaris* and *Avena sativa* by fermentation with the *Pleurotus ostreatus* fungus. *Molecules, 22*, 2275. https://doi.org/10.3390/molecules22122275
Galanakis, C., Goulas, V., Tsakona, S., Manganaris, G., & Gekas, V. (2013). A knowledge base for the recovery of natural phenols with different solvents. *International Journal of Food Properties, 16*(2), 382–396. https://doi.org/10.1080/10942912.2010.522750
Ganguly, S., Sabikhi, L., & Singh, A. K. (2021). Cereal-based fermented foods for enhanced nutritional attributes and better gut health. *International Journal of Fermented Food, 10*(01), 1–12. https://doi.org/10.30954/2321-712X.01.2021.1
Grosso, G. (2018). Dietary antioxidants and prevention of non-communicable diseases. *Antioxidants, 7*(7), Article 94. https://doi.org/10.3390/antiox7070094
Hadidi, M., Liñán-Atero, R., Tarahi, M., Christodoulou, M. C., & Aghababaei, F. (2024). The potential health benefits of gallic acid: Therapeutic and food applications. *Antioxidants, 13*(8), Article 1001. https://doi.org/10.3390/antiox13081001
Hasan, M. M., Islam, M. E., Hossain, M. S., Akter, M., Rahman, M. A. A., Kazi, M., Khan, S., & Parvin, M. S. (2023). Unveiling the therapeutic potential: Evaluation of anti-inflammatory and antineoplastic activity of *Magnolia champaca* Linn's stem bark isolate through molecular docking insights. *Heliyon, 10*(1), e22972. https://doi.org/10.1016/j.heliyon.2023.e22972
Holt, R. R., Munafo, J. P., Salme, J., Keen, C. L., Mistry, B. S., Whiteley, J. M., & Schmitz, H. H. (2024). Mycelium: A nutrient-dense food to help address world hunger, promote health, and support a regenerative food system. *Journal of Agriculture and Food Chemistry, 72*(5), 2697–2707. https://doi.org/10.1021/2Facs.jafc.3c03307
Hu, S., Yin, J., Nie, S., Wang, J., Phillips, G. O., Xie, M., & Cui, S. W. (2016). In vitro evaluation of the antioxidant activities of carbohydrates. *Bioactive Carbohydrates and Dietary Fibre, 7*(2), 19–27. https://doi.org/10.1016/j.bcdf.2016.04.001
Huang, S. J., Tsai, S. Y., Lin, S. Y., Liang, C. H., Lian, P. Y., & Mau, J. L. (2011). Preparation of culinary-medicinal maitake mushroom, *Grifola frondosa* (Dicks.: Fr.) S.F. Gray (Aphyllophoromycetideae)-fermented wheat and its antioxidant properties. *International Journal of Medicinal Mushrooms, 13*(1), 61–71. https://doi.org/10.1615/IntJMedMushr.v13.i1.80
Huynh, N. T., Camp, J. V., Smagghe, G., & Raes, K. (2014). Improved release and metabolism of flavonoids by steered fermentation process: A review. *International Journal of Molecular Sciences, 15*, 19369–19388. https://doi.org/10.3390/ijms151119369
Jaiswal, A., & Lakshmi, J. (2022). Maximizing the bioaccessibility of iron and zinc of a complementary food mix through multiple strategies. *Food Chemistry, 372*, Article 131286. https://doi.org/10.1016/j.foodchem.2021.131286
Jurkaninová, L., Dvořáček, V., Gregusová, V., & Havrlentová, M. (2024). Cereal β-d-glucans in food processing applications and nanotechnology research. *Foods, 13*(3), Article 500. https://doi.org/10.3390/foods13030500
Kassim, N. K., Rahmani, M., Ismail, A., Sukari, M. A., Cheng, G. L. E., Mohd Nasir, N., & Awang, K. (2013). Antioxidant activity-guided separation of coumarins and lignan from *Melicope glabra* (Rutaceae). *Food Chemistry, 139*(1–4), 87–92. https://doi.org/10.1016/j.foodchem.2013.01.108
Khan, A. N., Singh, R., Bhattacharya, A., Chakravarti, R., Roy, S., Ravichandiran, V., & Ghosh, D. (2022). A short review on glucogallin and its pharmacological activities. *Mini Reviews in Medicinal Chemistry, 22*(22), 2820–2830. https://doi.org/10.2174/1389557522666220513150907
Kim, M. E., & Lee, J. S. (2025). Advances in the regulation of inflammatory mediators in nitric oxide synthase: Implications for disease modulation and therapeutic approaches. *International Journal of Molecular Sciences, 26*(3), Article 1204. https://doi.org/10.3390/ijms26031204
Krishnen G., Khulidin K.A., Ibadallah, B.X., Khairudin, S., Othman, Z., Ten, S.T., Ridzuan, N.F.M., Hamid, M.H., & Hasan, M.N.A.A.J. (2021). Performance of Solid and Liquid Spawns in Split Gill Mushroom Production. Presentation at the Mushroom Science Virtual.
Kumar, A., Bharti, A. K., & Bezie, Y. (2022). *Schizophyllum commune*: A fungal cell-factory for production of valuable metabolites and enzymes. *BioResources, 17*(3), 5420–5436. https://doi.org/10.15376/biores.17.3. Kumar
Li, X., Tian, Y., Wang, T., Lin, Q., Feng, X., Jiang, Q., Liu, Y., & Chen, D. (2017). Role of the *p*-coumaroyl moiety in the antioxidant and cytoprotective effects of flavonoid glycosides: Comparison of astragalin and tiliroside. *Molecules, 22*(7), Article 1165. https://doi.org/10.3390/molecules22071165
Li, Y., Xu, L., & Guo, S. (2023). Effects of solid-state fermentation with the *Ganoderma* spp. and *Coriolus versicolor* on the total phenol contents and antioxidant properties on black soybean. *Journal of Chemistry*. https://doi.org/10.1155/2023/9462748
López-Cárdenas, F.G., Pérez-Jiménez, J., Mateos-Briz, R., Zamora-Gasga, V.M., Sánchez-Burgos, J.A., & Sáyago-Ayerdi, S.G. (2023). Advances in mangiferin: biosynthetic pathways, bioavailability and bioactivity. In: Xiao, J. (eds) Handbook of Dietary Flavonoids. Springer. https://doi.org/10.1007/978-3-030-94753-8_53-1
Ma, M.-L., Tang, Z.-L., Chen, L.-P., Qin, X.-N., Xiao, K.-F., Zhu, W.-L., Zhang, Y., & Gong, Z.-B. (2025). Cistanoside F ameliorates lipid accumulation and enhances myogenic differentiation via AMPK-dependent signalling in C2C12 myotubes. *Cells, 14*(12), Article 874. https://doi.org/10.3390/cells14120874
McCue, P., Horii, A., & Shetty, K. (2004). Mobilization of phenolic antioxidants from defatted soybean powders by *Lentinus edodes* during solid-state bioprocessing is associated with enhanced production of laccase. *Innovative Food Science & Emerging Technologies, 5*(3), 385–392. https://doi.org/10.1016/j.ifset.2004.05.003
Melini, F., & Melini, V. (2021). Impact of fermentation on phenolic compounds and antioxidant capacity of quinoa. *Fermentation, 7*(1), Article 20. https://doi.org/10.3390/fermentation7010020
Mihaylova, D., & Lante, A. (2019). Water an eco-friendly crossroad in green extraction: An overview. *The Open Biotechnology Journal, 13*, 155–162. https://doi.org/10.2174/1874070701913010155
Mišković, J., Rašeta, M., Krsmanović, N., & Karaman, M. (2023). Update on mycochemical profile and selected biological activities of genus *Schizophyllum* Fr. 1815. *Microbiology Research, 14*(1), 409–429. https://doi.org/10.3390/microbiolres14010031
Nehru, L., Kandasamy, G. D., Sekar, V., Alshehri, M. A., Panneerselvam, C., Alasmari, A., & Kathirvel, P. (2023). Green synthesis of ZnO-NPs using endophytic fungal extract of *Xylaria arbuscula* from *Blumea axillaris* and its biological applications. *Artificial Cells, Nanomedicine, and Biotechnology, 51*(1), 318–333. https://doi.org/10.1080/21691401.2023.2232654

Nirmal, N. P., & Panichayupakaranant, P. (2015). Antioxidant, antibacterial, and anti-inflammatory activities of standardized brazilin-rich *Caesalpinia sappan* extract. *Pharmaceutical Biology, 53*(9), 1339–1343. https://doi.org/10.3109/13880209.2014.982295
Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science and Nutrition, 6*(8), 2446–2458. https://doi.org/10.1002/fsn3.846
Patel, D. K., & Patel, K. (2023). An overview of medicinal importance, pharmacological activities and analytical aspects of fraxin from *Cortex Fraxinus*. *Current Traditional Medicine, 9*(5), Article e190922208921. https://doi.org/10.2174/2215083808666220919114652
Peng, B., Bai, R. F., Li, P., Han, X. Y., Wang, H., Zhu, C. C., Zeng, Z. P., & Chai, X. Y. (2015). Two new glycosides from *Dryopteris fragrans* with anti-inflammatory activities. *Journal of Asian Natural Products Research, 18*(1), 59–64. https://doi.org/10.1080/10286020.2015.1121853
Pérez-Chávez, A. M., Alberti, M. M., & Albertó, E. (2022). Evaluation of ligninolytic activity in spent mushroom substrate from four cultivated mushrooms. *Journal of Bioresources and Bioproducts, 7*(4), 288–294. https://doi.org/10.1016/j.jobab.2022.09.001
Piazza, S., Martinelli, G., Maranta, N., Pozzoli, C., Fumagalli, M., Nicolaci, V., Sonzogni, E., Colombo, L., Sangiovanni, E., & Dell'Agli, M. (2024). Investigation into the anti-acne effects of *Castanea sativa* mill leaf and its pure ellagitannin castalagin in HaCaT cells infected with *Cutibacterium acnes*. *International Journal of Molecular Sciences, 25*(9), 4764. https://doi.org/10.3390/ijms25094764
Sadh, P. K., Duhan, S., & Duhan, J. S. (2018). Agro-industrial wastes and their utilization using solid-state fermentation: A review. *Bioresources & Bioprocessing, 5*(1), Article 1. https://doi.org/10.1186/s40643-017-0187-z
Salek, R. M., Steinbeck, C., Viant, M. R., Goodacre, R., & Dunn, W. B. (2013). The role of reporting standards for metabolite annotation and identification in metabolomic studies. *Gigascience, 2*(1), Article 13. https://doi.org/10.1186/2047-217X-2-13
Santos, M. C. B., da Silva Lima, L. R., Nascimento, F. R., do Nascimento, T. P., Cameron, L. C., & Ferreira, M. S. L. (2019). Metabolomic approach for characterization of phenolic compounds in different wheat genotypes during grain development. *Food Research International, 124*, 118–182. https://doi.org/10.1016/j.foodres.2018.08.034
Šelo, G., Planinić, M., Tišma, M., Klarić, A.-M., & Bucić-Kojić, A. (2024). Effects of fungal solid-state fermentation on the profile of phenolic compounds and on the nutritional properties of grape pomace. *Microorganisms, 12*(7), Article 1310. https://doi.org/10.3390/microorganisms12071310
Sevgi, K., Tepe, B., & Sarikurkcu, C. (2015). Antioxidant and DNA damage protection potentials of selected phenolic acids. *Food and Chemical Toxicology, 77*, 12–21. https://doi.org/10.1016/j.fct.2014.12.006
Shabbir, U., Tyagi, A., Ham, H. J., Elahi, F., & Oh, D. H. (2022). Effect of fermentation on the bioactive compounds of the black soybean and their anti-Alzheimer's activity. *Frontiers in Nutrition, 9*, 880361. https://doi.org/10.3389/2Ffnut.2022.880361
Silva, M., Ramos, A. C., Lidon, F. J., Reboredo, F. H., & Gonçalves, E. M. (2024). Pre-and postharvest strategies for *Pleurotus ostreatus* mushroom in a circular economy approach. *Foods, 13*(10), Article 1464. https://doi.org/10.3390/foods13101464
Singh, S. K., Kaldate, R., & Bisht, A. (2022). Citric acid, antioxidant effects in health. In S. M. Nabavi & A. S. Silva (Eds.), *antioxidant effects in health* (pp. 309–332). Elsevier. https://doi.org/10.1016/B978-0-12-819096-8.00045-8
Song, T., Zhang, Z., Jin, Q., Feng, W., Shen, Y., Fan, L., & Cai, W. (2021). Nutrient profiles, functional compositions, and antioxidant activities of seven types of grain fermented with *Sanghuangporus sanghuang* fungus. *Journal of Food Science and Technology, 58*, 4091–4101. https://doi.org/10.1007/s13197-020-04868-7
Subramaniam, S., Sabaratnam, V., Kuppusamy, U. R., & Tan, Y. S. (2014). Solid-substrate fermentation of wheat grains by mycelia of indigenous species of the genus *Ganoderma* (higher Basidiomycetes) to enhance the antioxidant activities. *International Journal of Medicinal Mushrooms, 16*(3), 259–267. https://doi.org/10.1615/intjmedmushr.v16.i3.60
Sułkowska-Ziaja, K., Korczyński, M., Trepa, M., Galanty, A., Lazur, J., Kubica, P., Kała, K., Paśko, P., & Muszyńska, B. (2025). A comparative study on the mycelium and fruiting body of *Meripilus giganteus*: Chemical composition and biological activity. *Current Issues in Molecular Biology, 47*(5), Article 302. https://doi.org/10.3390/cimb47050302
Tanaskovic, S.J., Šekuljica, N., Jovanovic, J., Gazikalovic, I., Grbavcˇic, S., Đord ē vic, N., Sekulic ́, M.V., Hao, J., Lukovic, N., & Kneževic-Jugovic, Z. (2021). Upgrading of valuable food component contents and anti-nutritional factors depletion by solid-state fermentation: a way to valorize wheat bran for nutrition. *Journal of Cereal Science*, *99*, 103159. https://doi.org/10.1016/j.jcs.2020.103159
Tiangpook, S., Nhim, S., Prangthip, P., Pason, P., Tachaapaikoon, C., Ratanakhanokchai, K., & Waeonukul, R. (2023). Production of a series of long-chain isomaltooligosaccharides from maltose by *Bacillus subtilis* AP-1 and associated prebiotic properties. *Foods, 12*(7), Article 1499. https://doi.org/10.3390/foods12071499
Tovar-Herrera, O. E., Martha-Paz, A. M., Pérez-Llano, Y., Aranda, E., Tacoronte-Morales, J. E., Pedroso-Cabrera, M. T., Arévalo-Niño, K., Folch-Mallol, J. L., & Batista-Garcia, R. A. (2018). *Schizophyllum commune*: An unexploited source for lignocellulose degrading enzymes. *MicrobiologyOpen, 7*, e637. https://doi.org/10.1002/mbo3.637
Tsafrakidou, P., Michaelidou, A.-M., & G. Biliaderis, C. (2020). Fermented cereal-based products: Nutritional aspects, possible impact on gut microbiota and health implications. *Foods, 9*(6), Article 734. https://doi.org/10.3390/foods9060734
Vignesh, A., Amal, T. C., Sarvalingam, A., & Vasanth, K. (2024). A review on the influence of nutraceuticals and functional foods on health. *Food Chemistry Advances, 5*, 100749. https://doi.org/10.1016/j.focha.2024.100749
Wang, J., Jiang, Q., Huang, Z., Wang, Y., Roubik, H., Yang, K., Cai, M., & Sun, P. (2023). Solid-state fermentation of soybean meal with edible mushroom mycelium to improve its nutritional, antioxidant capacities and physicochemical properties. *Fermentation, 9*, 322. https://doi.org/10.3390/fermentation9040322
Widowati, W., Kusuma, H. S. W., Arumwardana, S., Afifah, E., Wahyuni, C. D., Wijayanti, C. R., Maulana, M. A., & Rizal, R. (2021). Corilagin potential in inhibiting oxidative and inflammatory stress in LPS-induced murine macrophage cell lines (RAW 264.7). *Iranian Journal of Basic Medical Sciences, 24*(12), 1656–1665. https://doi.org/10.22038/IJBMS.2021.59348.13174
Xu, X., Liu, Y., Pan, C., Han, S., Ma, L., Qiao, Y., Shi, B., & Peng, Q. (2023). Antioxidant and immunomodulatory activities of polysaccharides from fermented wheat products of *Grifola frondosa*: *In vitro* methods. *International Journal of Food Science, 9*, 3820276. https://doi.org/10.1155/2F2023/2F3820276
Yang, Y., Liang, Y., Cui, F., Wang, Y., Sun, L., Zan, X., & Sun, W. (2023). UDP-glycosyltransferases in edible fungi: Function, structure, and catalytic mechanism. *Fermentation, 9*(2), Article 164. https://doi.org/10.3390/fermentation9020164
Zhai, F. H., Wang, Q., & Han, J. R. (2015). Nutritional components and antioxidant properties of seven kinds of cereals fermented by the basidiomycete *Agaricus blazei*. *Journal of Cereal Science, 65*, 202–208. https://doi.org/10.1016/j.jcs.2015.07.010
Zhang, Z., Lv, G., Pan, H., Fan, L., Soccol, C. R., & Pandey, A. (2012). Production of powerful antioxidant supplements via solid-state fermentation of wheat (*Triticum aestivum* Linn.) by *Cordyceps militaris*. *Food Technology and Biotechnology, 50*(1), 31–39.
Zhang, J., Jiang, Z., Wei, W., Li, X., Sun, C., Zhang, Y., Fu, S., & Zheng, J. (2019). Target elimination-denatured and unstable proteins, environmental toxins, metabolic wastes, immunosuppressive factors and chronic inflammatory factors of medical system for chronic diseases prevention and health promotion: A narrative review. *Iranian Journal of Public Health, 48*(6), 994–1003.
Zhang, J., Liu, M., Zhao, Y., Zhu, Y., Bai, J., Fan, S., Zhu, L., Song, C., & Xiao, X. (2022). Recent developments in fermented cereals on nutritional constituents and potential health benefits. *Foods, 11*, Article 2243. https://doi.org/10.3390/foods11152243
Zhao, Y.-S., Eweys, A. S., Zhang, J.-Y., Zhu, Y., Bai, J., Darwesh, O. M., Zhang, H.-B., & Xiao, X. (2021). Fermentation affects the antioxidant activity of plant-based food material through the release and production of bioactive components. *Antioxidants, 10*(12), Article 2004. https://doi.org/10.3390/antiox10122004

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